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MINIREVIEW

Three Classes of Cell Surface Receptors for Alphaherpesvirus Entry

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Herpesviruses have been classified into three subfamilies on the basis of biological characteristics and genomic analysis. Members of the alphaherpesvirus subfamily are neurotropic, have a short replicative cycle, and, in general, have a broad host range. In addition, they encode a similar set of homologous genes arranged in similar order. Also, selected proteins of one alphaherpesvirus may functionally substitute for the homolog of another.

Human herpes simplex viruses 1 and 2 (HSV-1 and HSV-2), porcine pseudorabies virus (PRV), and bovine herpesvirus 1 (BHV-1) are representative members of the alphaherpesvirus subfamily and are the subject of this review. Natural diseases associated with infections by these viruses are restricted to specific hosts. However, these viruses can infect selected laboratory animals, such as rodents, and all have a relatively broad host range for cultured cells, at least for entry. The obvious inferences are that each of these viruses can use multiple cell surface receptors for entry or that each can recognize structural features of receptors conserved among human and animal species. The evidence summarized here shows that both inferences are correct.

The usual manifestations of HSV disease are lesions on mucosal epithelium (oral or genital), skin, or cornea; latent infection of neurons in sensory ganglia; and perhaps recurrent lesions at the site of primary infection, due to reactivation of latent virus from the ganglia. Encephalitis can occur, albeit rarely in children or adults; newborn infants can suffer from severe disseminated disease. PRV and BHV-1 can cause similar manifestations of disease in pigs and cattle. Thus, important cellular targets of infection include cells of the mucosal epithelium and neurons, but are not limited to these cell types.

The focus of this review is cell surface receptors for entry of alphaherpesviruses into cells. The pathway of entry is via fusion of the virion envelope with a cell membrane, often the cell plasma membrane. Membrane fusion induced by alphaherpesviruses does not require activation by low pH but depends upon multiple interactions of viral proteins with cell surface components. Figure 1 identifies components of the viral envelope and cell membrane that govern viral entry and are described below. The information summarized here probably applies to most alphaherpesviruses except for those, such as varicella-zoster virus, that apparently lack a gene encoding gD, a viral glycoprotein central to the entry process for HSV, PRV, and BHV-1. Citations listed at the end are mostly recent publications related to cell receptors for these viruses; reviews should be consulted for earlier work.

The components of a herpesvirus particle are its DNA core, icosahedral capsid, tegument (proteins located between the capsid surface and envelope), and envelope, which is a lipid bilayer containing multiple viral membrane proteins and glycoproteins.

The alphaherpesvirus envelope contains about a dozen viral membrane proteins, only five of which have defined roles in viral entry. For HSV-1, PRV, and BHV-1, binding of virus to cells is mediated principally by gC, although other glycoproteins such as gB and gD can contribute to stable binding or can substitute for gC in mediating binding. Penetration of virus requires fusion of the virion envelope with a cell membrane and depends on the action of gB, gD, gH, and gL. Deletion of the genes for any one of these four glycoproteins is lethal and results in production of virions that can bind to cells, provided gC is present, but cannot penetrate. Interestingly, deletion of gC is not a lethal mutation, although it

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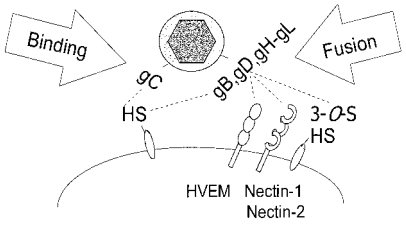

FIG. 1. Molecular determinants of HSV entry into cells. Five of the dozen or so membrane glycoproteins in the viral envelope have defined roles in viral entry. The initial binding of virus to cell surface heparan sulfate (HS) can be mediated by viral gC or gB. This facilitates the binding of gD to any one of several cell surface receptors. These include HVEM, a member of the TNF receptor family, nectin-1 or nectin-2, both members of the immunoglobulin superfamily, or specific sites in heparan sulfate (3-O-S) generated by the action of specific 3-O-sulfotransferases. The binding of gD to one of these receptors can then trigger fusion between the virion envelope and cell membrane. This fusion requires the concerted action of gB, gD, gH, and gL. Other alphaherpesviruses express related glycoproteins with similar roles in entry and can also use nectins as gD receptors for entry.

can cause reduced binding and infectivity [see reviews by Mettenleiter (2000) and Spear (1993a) for citations documenting these findings].

For PRV and BHV-1, at least, the lethal effects of deleting gD can be partially reversed by secondary mutations that enable infectious virus to be produced in the absence of gD (Schmidt *et al.,* 1997; Schröder *et al.,* 1997). The secondary mutations are probably multiple and remain to be fully defined but may include a mutation in gH, at least in the case of BHV-1 (Schröder and Keil, 1999). These results suggest that gD is not necessarily an essential part of the membrane-fusing machinery, although it is the viral ligand for cell surface receptors that mediate viral entry, as discussed below.

The initial interaction of HSV-1, HSV-2, PRV, and BHV-1 with cells is usually binding of virion gC to cell surface glycosaminoglycans, preferentially heparan sulfate (Mettenleiter *et al.,* 1990; Okazaki *et al.,* 1991; Shieh *et al.,* 1992; WuDunn and Spear, 1989). The importance of heparan sulfate as a cell determinant of viral entry is underscored by the results of selecting for cell mutants that are resistant to the cytotoxic effects of HSV. Such mutants have been isolated from mouse L cells (Banfield *et al.,* 1995; Gruenheid *et al.,* 1993) and Chinese hamster ovary (CHO) cells (Wei *et al.,* 2000) and, in both cases, were defective for heparan sulfate biosynthesis and partially or substantially resistant to viral entry. The ability of alphaherpesviruses to bind to cells via interactions with heparan sulfate is a feature of clinical isolates as well as laboratory strains. It is not a property acquired by the passage of virus in cultured cells (Terhune *et al.,* 1998).

Neither gC nor heparan sulfate is absolutely required for entry of virus into cells. In the absence of gC, all of the alphaherpesviruses described here can infect cells, although the efficiency of binding and entry may be severely reduced (Herold *et al.,* 1991; Mettenleiter, 1989; Okazaki *et al.,* 1987). In the case of HSV, gB can mediate the binding of virus to heparan sulfate if gC is absent (Herold *et al.,* 1994). In the case of PRV and BHV-1, it is not known how gC-negative mutants bind to cells but the binding appears not to be dependent on heparan sulfate (Karger *et al.,* 1995; Klupp *et al.,* 1997). In the absence of heparan sulfate and other glycosoaminoglycans to which virus can bind, entry is very inefficient but can occur, probably through the interaction of gD with cell surface receptors.

Binding of virus to heparan sulfate does not necessarily lead to alphaherpesvirus entry. HSV-1, PRV, and BHV-1 can bind to heparan sulfate on certain cell types, such as CHO cells, but fail to penetrate (Geraghty *et al.,* 1998; Shieh *et al.,* 1992). The existence of cells that allowed virus binding, but resisted entry, facilitated the expression cloning of cDNAs encoding human and mouse proteins capable of converting resistant cells to susceptibility to HSV entry. Heparan sulfate and the new receptors identified by expression cloning will be referred to as coreceptors for entry because, in general, alphaherpesvirus entry is facilitated by, if not dependent on, interactions of viral proteins with at least two, perhaps more, cell surface receptors.

Table 1 lists the human and rodent proteins shown to serve as coreceptors, or to generate coreceptors, for the entry of HSV and other alphaherpesviruses. The first HSV entry receptor to be identified was a previously unrecognized human member of the tumor necrosis factor (TNF) receptor family, designated herpesvirus entry mediator (HVEM) originally (Montgomery *et al.,* 1996) and later herpesvirus entry protein A (HveA) (Warner *et al.,* 1998) and catalogued as TNFRSF14. Additional human entry receptors were identified (Cocchi *et al.,* 1998; Geraghty *et al.,* 1998; Warner *et al.,* 1998) and proved to be related members of the immunoglobulin superfamily, a subfamily including the poliovirus receptor (CD155) (Mendelsohn *et al.,* 1989) and two proteins that are not receptors for poliovirus but were originally designated poliovirus receptor-related protein-1 (Prr1) (Lopez *et al.,* 1995) and poliovirus receptor-related protein 2 (Prr2) (Eberlé *et al.,* 1995) and were renamed HveC (Geraghty *et al.,* 1998) and HveB (Warner *et al.,* 1998), respectively. Recently both HveB and HveC were shown to be homophilic cell adhesion molecules that localize to sites of cadherin-based cell junctions (Aoki *et al.,* 1997; Lopez *et al.,* 1998; Satoh-Horikawa *et al.,* 2000; Takahashi *et al.,* 1999) and were renamed nectin-2 (HveB) and nectin-1 (HveC). CD155 and the nectins are expressed as multiple isoforms differing at their C-termini (i.e., nectin-1α and nectin-1β). Alphaherpesvirus entry activity is not influenced by these differences provided the protein is membrane-bound. Nomenclature for these viral entry receptors is in a state of flux. Here we have chosen to use the name HVEM, rather than HveA, because the former name is more widely used in the literature, and to use

TABLE 1

Human and Rodent Proteins That Serve as gD Receptors, or Generate gD Receptors, for Alphaherpesvirus Entry

Entry mediator			Mediates entry of				
			HSV-1				
Protein family	Name used here	Other names	WT	Rid[a]	HSV-2	PRV	BHV-1
TNF receptor	HVEM	ATAR; TR2; HveA; TNFRSF-14	***Hu-Yes***[b] ***Mu-Yes***	*Hu-No* ***Mu-Yes***	***Hu-Yes*** ***Mu-Yes***	*Hu-No* *Mu-No*	*Hu-No* *Mu-No*
Related subfamily of the immunoglobulin superfamily	Nectin-1α	Prr1α; HveC	***Hu-Yes*** ***Mu-Yes***	***Hu-Yes*** ***Mu-Yes***	***Hu-Yes*** ***Mu-Yes***	***Hu-Yes*** ***Mu-Yes***	***Hu-Yes*** ***Mu-Yes***
	Nectin-1β	Prr1β; HIgR	***Hu-Yes***	***Hu-Yes***	***Hu-Yes***	Hu-NT	***Hu-Yes***
	Nectin-2α	Prr2α; HveB; Mphα	*Hu-No* *Mu-No*	***Hu-Yes*** *Mu-No*	***Hu-Yes*** *Mu-No*	***Hu-Yes*** ***Mu-Yes***	*Hu-No* *Mu-No*
	Nectin-2δ	Prr2δ; Mphβ	*Hu-No* Mu-NT	***Hu-Yes*** Mu-NT	Hu-NT Mu-NT	Hu-NT Mu-NT	Hu-NT Mu-NT
	CD155	Pvr; Tage4[c]	*Hu-No* *Mu-No* *Ra-No*	*Hu-No* *Mu-No* *Ra-No*	*Hu-No* *Mu-No* *Ra-No*	***Hu-Yes*** *Mu-No* ***Ra-Yes***	***Hu-Yes*** *Mu-No* ***Ra-Yes***
Sulfotransferase	3-OST-3_A; 3-OST-3_B		***Hu-Yes*** ***Mu-Yes***	*Hu-No* *Mu-No*	*Hu-No* *Mu-No*	*Hu-No* *Mu-No*	*Hu-No* *Mu-No*

[a] Mutants of HSV-1 that are *r*esistant to *i*nterference mediated by wild-type HSV-1 g*D* (Rid) and have amino acid substitutions at position 25 or 27 of gD.

[b] For various human (Hu), mouse (Mu), or rat (Ra) orthologs of the proteins indicated, "Yes" and "No" indicate that the protein has been tested for entry activity and is positive or not for each virus; "NT" means "not tested" or not reported.

[c] Rat and mouse proteins named Tage4 are proposed to be the rodent orthologs of CD155, the poliovirus receptor (B. Baury, D. Masson, P. Lustenberger, R. J. Geraghty, P. G. Spear, and M. G. Denis, manuscript in preparation).

nectin-1 and nectin-2, rather than HveC and HveB, because the former names are based on cellular function.

A newly discovered HSV-1 entry receptor is generated in heparan sulfate by the action of specific glucosaminyl 3-O-sulfotransferases. In the absence of other entry receptors described above, resistant cells can be made susceptible to HSV-1 entry, but not entry of HSV-2, PRV, or BHV-1, by expression of 3-O-sulfotransferase-3, provided cell surface heparan sulfate is present (Shukla *et al.,* 1999a).

Several remarkable findings about these coreceptors should be emphasized. First, at least three classes of cell surface molecules can independently serve as coreceptors, along with sites recognized by gC in heparan sulfate, for HSV entry. Second, human and mouse (Menotti *et al.,* 2000; Shukla *et al.,* 1999b, 2000) versions of these proteins have similar, but not identical, entry activities for the alphaherpesviruses listed in Table 1. Third, available evidence indicates that all are receptors for viral gD (Cocchi *et al.,* 1998; Geraghty *et al.,* 2000; Krummenacher *et al.,* 1998; Nicola *et al.,* 1998; Whitbeck *et al.,* 1997), except for the 3-O-sulfotransferases (3-OST-3s) that generate gD receptors in heparan sulfate (Shukla *et al.,* 1999a). Fourth, the affinities of these diverse receptors for gD appear to be similar with K_D values in the micromolar range, at least for the cases of HSV gD binding to HVEM, nectin-1, and 3-OST-3-modified heparan sulfate (Krummenacher *et al.,* 1999; Rux *et al.,* 1998; Shukla *et al.,* 1999a; Willis *et al.,* 1998). Fifth, because sites in heparan sulfate recognized by gC are present on many cell types whereas the gD receptors have a more restricted distribution, the presence of appropriate gD receptors appears to govern the susceptibility of cells to alphaherpesvirus entry. Sixth, single amino acid substitutions in gD can result in loss of ability to use one receptor and acquisition of ability to use another. For example, wild-type HSV-1 strains can enter cells via HVEM, but not human nectin-2, whereas Rid mutants, which differ from the wild type by an amino acid substitution at position 25 or 27 in gD, can use human nectin-2 but not human HVEM (Table 1 and Fig. 2) (Lopez *et al.,* 2000; Warner *et al.,* 1998). Seventh, some of the gD receptors, such as human or mouse nectin-1 (Geraghty *et al.,* 1998; Menotti *et al.,* 2000; Shukla *et al.,* 2000), can serve as entry coreceptors for all the alphaherpesviruses discussed here, whereas others that were identified on the basis of entry activity for HSV-1 are specific for HSV strains (Montgomery *et al.,* 1996; Shukla *et al.,* 1999a).

The divergence of gD primary sequences among the animal and human alphaherpesviruses (Table 2) must

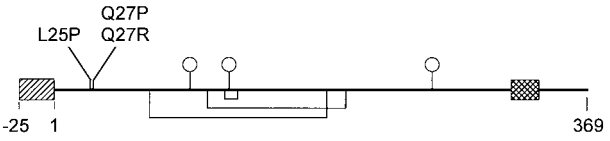

FIG. 2. Molecular features of wild-type HSV-1 gD and mutant forms that have altered receptor preferences. A 25-amino-acid signal peptide (hatched box) is cleaved from the translation product to yield a 369-amino-acid type 1 membrane protein with its transmembrane domain (cross-hatched box) located near the C-terminus. Three N-linked glycans are located at the positions of the lollipops; the protein also acquires O-linked glycans. The six Cys residues in the ectodomain are covalently linked in the disulfide-bonding pattern indicated by the lines drawn under the polypeptide chain. These Cys residues are conserved in all the alphaherpesvirus gDs. Viruses with amino acid substitutions at position 25 or 27, as indicated, are resistant to interference mediated by wild-type gD (Rid mutants) resulting from their altered receptor preferences. They can enter cells via nectin-2, which is not an effective entry receptor for wild-type HSV-1. See review by Cohen *et al.* (1992) for citations supporting the structural information summarized here.

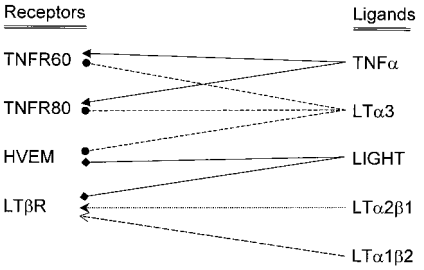

FIG. 3. Four members of the TNF receptor family and their ligands. Most of the ligands can interact with more than one receptor and multiple receptors can respond to the same ligand (reviewed by Wallach *et al.,* 1999).

permit conservation of structural features important for universal binding to nectin-1 while allowing selective interactions with other gD receptors. The gD receptors with broad specificity for multiple human and animal viruses can explain the broad host range of alphaherpesviruses, at least with respect to entry. Those with more limited specificity may explain, at least in part, biological differences among the various alphaherpesviruses, including the ability to cause natural disease in different human or animal hosts. The fact that single amino acid substitutions in gD can alter receptor preferences mandates a search for natural gD polymorphisms that might influence cell tropism and hence pathogenesis. The fact that most of the viruses listed in Table 1 use homologous receptors for entry into mouse and human cells enhances the utility of mouse models of disease for studying aspects of pathogenesis dependent on susceptibility of cells to viral entry.

HVEM is expressed in lymphoid cells but also in other cell types (Kwon *et al.,* 1997; Montgomery *et al.,* 1996). Mice encode a related protein that is only 45% identical to the human form (Hsu *et al.,* 1997). Both human and mouse forms of HVEM can mediate entry of HSV-1 and HSV-2 strains (except that human HVEM is unable to mediate entry of HSV-1 Rid mutants) but have no entry activity for PRV or BHV-1 (Table 1) (Montgomery *et al.,* 1996; D. Shukla and P. G. Spear, manuscript in preparation). It remains to be determined whether porcine or bovine proteins homologous to HVEM might be entry receptors for the animal viruses.

Natural ligands for HVEM are members of the TNF family including lymphtoxin-α and LIGHT (Mauri *et al.,* 1998). Both can also serve as ligands for other members of the TNF receptor family, as diagrammed in Fig. 3. The cytoplasmic tail of HVEM can transmit signals through TNF receptor-associated factors (TRAFs) resulting, for example, in activation of NF-κB and AP-1 (Hsu *et al.,* 1997; Marsters *et al.,* 1997). LIGHT can stimulate the proliferation of T cells, presumably through interactions with HVEM (Harrop *et al.,* 1998; Kwon *et al.,* 1997). LIGHT can also induce apoptosis of tumor cells expressing both HVEM and LTβR (Harrop *et al.,* 1998; Zhai *et al.,* 1998), but the apoptotic activity depends on the LIGHT–LTβR interaction (Rooney *et al.,* 2000). Deletion of the cytoplasmic tail from HVEM to remove all TRAF-binding sequences has no effect on the ability of HVEM to mediate HSV entry (Montgomery *et al.,* 1996). Thus, signal transduction seems not to be required for viral entry, although signal transduction might occur when HSV enters a cell

TABLE 2

Amino Acid Sequence Identities in Pairwise Comparisons of Alphaherpesvirus gDs

Origin of gD[a]	Number of amino acids[b]	Percent identity[c] with			
		HSV-1 gD	HSV-2 gD	PRV gD	BHV-1 gD
HSV-1	394	(100)	82.5	25.4	28.4
HSV-2	393		(100)	25.6	29.3
PRV	400			(100)	38.5
BHV-1	417				(100)

[a] Strains selected for comparison were HSV-1 (KOS), HSV-2 (333), PRV (Kaplan), and BHV-1 (Cooper).

[b] Lengths of the primary translation products.

[c] Percent identity determined after alignment by the method of Hein (1990).

via full-length HVEM and thus influence downstream events in viral replication or virus–cell interaction.

Nectin-1 and nectin-2 are members of a small subfamily of the immunoglobulin superfamily as described above. This subfamily also includes CD155, the poliovirus receptor (Mendelsohn *et al.,* 1989), and nectin-3 (Satoh-Horikawa *et al.,* 2000). The human and mouse forms of nectin-1 have the broadest specificity for mediating alphaherpesvirus entry (Table 1) and are highly conserved in primary sequence (95% identity). It seems likely that various mammalian forms of nectin-1 will prove to be pan-alphaherpesvirus entry receptors.

Nectin-2 is more limited in its ability to mediate alphaherpesvirus entry (Lopez *et al.,* 2000; Shukla *et al.,* 1999b; Warner *et al.,* 1998). It is of interest that human nectin-2 is a coreceptor for HSV-2 entry but not for most HSV-1 strains unless they have amino acid substitutions at position 25 or 27 of gD. It will be important to learn whether nectin-2, with its specificity for one serotype, contributes to differences in the biology of HSV-1 and HSV-2.

It has been difficult to identify rodent homologs of CD155. The most likely candidates are rat and mouse Tage4, originally cloned on the basis of their overexpression in colon cancer (Chadeneau *et al.,* 1994; Chadeneau *et al.,* 1996). Interestingly, both CD155 and rat Tage4 are entry receptors for PRV and BHV-1 but not for HSV strains (Table 1) (Geraghty *et al.,* 1998; B. Baury, D. Masson, P. Lustenberger, R. J. Geraghty, P. G. Spear, and M. G. Denis, manuscript in preparation). To date, only the mouse version of nectin-3 has been sequenced and it is not known whether it is an alphaherpesvirus entry receptor.

Nectin-1, nectin-2, and nectin-3 are homotypic cell adhesion molecules (Aoki *et al.,* 1997; Lopez *et al.,* 1998; Satoh-Horikawa *et al.,* 2000; Takahashi *et al.,* 1999). Nectin-3 can also engage in heterotypic interactions with nectin-1 and nectin-2 (Satoh-Horikawa *et al.,* 2000). All members of this subfamily are expressed, through differential splicing of primary transcripts, as multiple isoforms that differ in their membrane-spanning and cytoplasmic domains or that lack a membrane-spanning domain. Some isoforms of nectin-1, nectin-2, and nectin-3 can localize to cadherin-based adherens junctions in epithelial cells through binding of their cytoplasmic tails to PDZ domains in afadin, a cytoplasmic protein that binds to actin filaments and components of cadherin-based cell junctions (Asakura *et al.,* 1999; Mandai *et al.,* 1997; Miyahara *et al.,* 2000; Satoh-Horikawa *et al.,* 2000; Takahashi *et al.,* 1999). This localization is not required for HSV entry because deletion of the cytoplasmic tail of nectin-1α is without effect on viral entry (R. J. Geraghty and P. G. Spear, unpublished results) and an isoform of nectin-1 that lacks the PDZ-binding cytoplasmic tail has entry activity (Cocchi *et al.,* 1998). Localization of nectin-1 and nectin-2 to junctions in epithelial cells and homophilic or heterophilic interactions could very well influence the availability of receptor for interactions with virus. Neuronal cells in the nervous system of mice also express nectin-1 (Shukla *et al.,* 2000; L. Haarr, D. Shukla, M. Dal Canto, and P. G. Spear, manuscript in preparation). It will be of interest to determine whether neuronal nectin-1 is localized to synapses, given the ability of alphaherpesviruses to be transmitted across synapses in the nervous system.

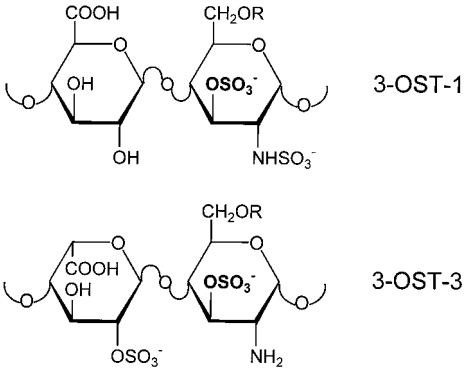

FIG. 4. Disaccharides that form part of specific protein binding sites in heparan sulfate. The site generated by 3-OST-1 is present in a pentasaccharide that binds antithrombin and the site generated by 3-OST-3 is present in a saccharide of unknown size that binds HSV-1 gD and serves as an entry receptor for HSV.

Specific sites in heparan sulfate generated by 3-O-sulfotransferases (3-OSTs) can serve as receptors for gD and as entry receptors for HSV-1 in the absence of any known protein receptors (Shukla *et al.,* 1999a). Expression of mouse or human 3-OST-3_A or 3-OST-3_B in resistant cells can render the cells susceptible to HSV-1 entry, but not to entry of the other viruses discussed here. The enzymes do not serve as entry receptors themselves but rather modify heparan sulfate to generate sites for the binding of gD. The receptors generated by the enzymes can be removed from cells by the action of heparitinases, which render the cells resistant to HSV-1 entry.

Heparan sulfate is constructed by a copolymerase that adds alternating residues of glucuronic acid and *N*-acetylglucosamine to a core oligosaccharide attached to a core protein (Lind *et al.,* 1998; Wei *et al.,* 2000). The polymer of repeating disaccharides is then modified sequentially by several sulfotransferases and an epimerase that converts some glucuronic acid residues to iduronic acid (reviewed by Lindahl *et al.,* 1998). Multiple genes encode distinct forms of each sulfotransferase, which can differ in substrate specificity. Differential expression of the various isoforms can lead to distinct patterns of specific binding sites for proteins, determined by specific patterns of sulfation and epimerization of uronic acid residues. One of the final modifications of heparan sulfate chains is sulfation of the hydroxyl group at the 3 position of the amino sugar, a reaction catalyzed by 3-OSTs. Figure 4 shows specific disaccharides that

result from the action of two different isoforms of 3-OST. The disaccharide generated by 3-OST-1 is a part of the specific pentasaccharide that forms the binding site for antithrombin (Lindahl *et al.,* 1979), whereas the disaccharide generated by 3-OST-3_A or 3-OST-3_B forms part of the specific binding site for gD (Liu *et al.,* 1999a,b; Shukla *et al.,* 1999a). Expression of the latter enzymes, but not of 3-OST-1, generates entry receptors for HSV-1. There are other isoforms of the 3-OSTs that remain to be tested for viral entry activity.

Alphaherpesvirus proteins induce membrane fusion during viral entry into cells and also in the context of virus-induced cell fusion. The HSV proteins required for viral entry and for cell–cell fusion are the same, gB, gD, gH, and gL (reviewed by Spear, 1993b; Turner *et al.,* 1998). Cell receptors are required for cell–cell fusion (Terry-Allison *et al.,* 1998, 2000) as well as for viral entry. The cell fusion receptors identified to date are the gD receptors for viral entry listed in Table 1. As mentioned above, gD may not be an essential component of the membrane-fusing machinery because mutations in the PRV or BHV-1 genomes can allow infectious virus to be produced in the absence of gD. However, gD, through its interaction with a cell surface receptor, apparently provides the usual trigger for initiating membrane fusion. One possibility is that the gD-receptor interaction alters the conformation of gD so that it interacts with gB and/or gH-gL heterodimers to activate the fusogenic activity of one or both. The components of the actual membrane-fusing machinery are not yet known so that any predictions about structural correlates of fusion activity would be premature.

Given that multiple cell surface receptors can be used by HSV to enter cells, which ones are actually used by HSV-1 and HSV-2 to infect epithelial cells at the portal of entry and to spread to other cells and tissues, including neuronal cells? Second, are differences in the epidemiology, natural history, or pathogenesis of HSV-1 and HSV-2 infections explained in part by the fact that members of the two serotypes differ somewhat in receptor preferences (Table 1)?

These questions remain to be answered. Obtaining the information needed will require a number of approaches. It is only by culturing cells *in vitro* that virus infections can be performed so as to identify the relevant receptors used for entry. However, the process of dissociating and culturing cells is likely to change the expression of a variety of genes, including those encoding HSV receptors or enzymes capable of generating receptors. Careful comparisons of receptor expression by relevant cells *in situ* and after *in vitro* culture can address this problem. Recognizing the pitfalls of *in vitro* studies, primary cells cultured from the appropriate tissues can be tested for susceptibility to HSV-1 or HSV-2 infection and reagents capable of blocking infection via a specific receptor tested for ability to block infection. For example, primary cultures of human T lymphocytes are susceptible to HSV-1 infection, which can be substantially inhibited by an antiserum specific for HVEM, indicating that HVEM is the primary gD receptor used for viral entry (Montgomery *et al.,* 1996).

In the case of cell types that express multiple HSV-1 or HSV-2 receptors, one possibility is that any of the receptors could mediate viral entry. Alternatively, in organized tissues, only one of several expressed receptors may be accessible for interaction with virus. For example, if the nectin isoforms expressed are localized to cell junctions in epithelial cells, they may be unavailable for binding to incoming virus. Thus, it becomes important to know which entry receptors are presented on the regions of the cell surface to which virus can bind.

Because the entry of HSV into human and mouse cells is largely via homologous receptors, mouse models of HSV infection can be exploited to provide information about viral entry. Blocking reagents specific for the various gD receptors might be used to identify receptors used by virus for infection of exposed body surfaces such as the cornea or mucosal epithelium. Mouse mutants deleted for specific genes or unable to express genes for particular gD receptors in particular cell types might also prove useful. Finally, it is possible to produce HSV mutants with altered receptor preferences (Table 1). Additional mutants of this type might also help to define the virus–receptor interactions that are important for the pathogenesis of wild-type virus.

The discovery of multiple HSV receptors belonging to three classes of cell surface molecules has changed the way we must think about investigating the requirements for viral entry into cells and the contribution to viral pathogenesis of cell susceptibility to entry.

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